Protein turnover; selective degradation/cleavage

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Individual cellular proteins turn over (are degraded and re-synthesized) at different rates.

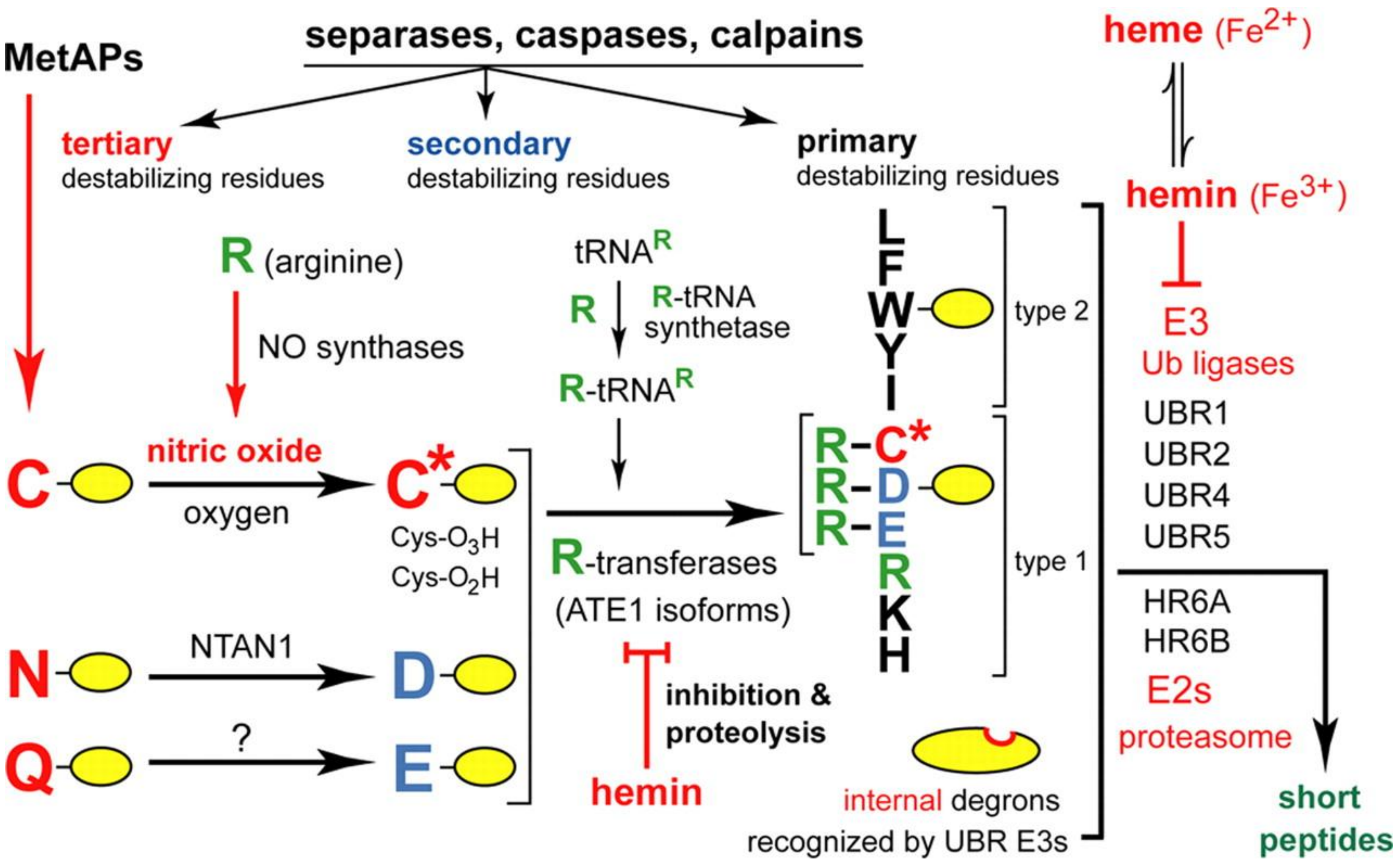
E.g., half-lives of selected enzymes of rat liver cells range from 0.2 to 150 hours.

N-end rule: On average, a protein's half-life correlates with its **N-terminal residue**.

- Proteins with N-terminal Met, Ser, Ala, Thr, Val, or Gly have half lives greater than 20 hours.
- Proteins with N-terminal Phe, Leu, Asp, Lys, or Arg have half lives of 3 min or less.

PEST proteins having domains rich in Pro (P), Glu (E), Ser (S), Thr (T), are more rapidly degraded than other proteins.

The mammalian N-end rule pathway.



Varshavsky A J. Biol. Chem. 2008;283:34469-34489

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- **Most autophagy** is **not** a mechanism for **selective** degradation of individual macromolecules.
- However, cytosolic proteins that include the sequence **KFERQ** may be **selectively** taken up by lysosomes in a process called **chaperone-mediated autophagy**.

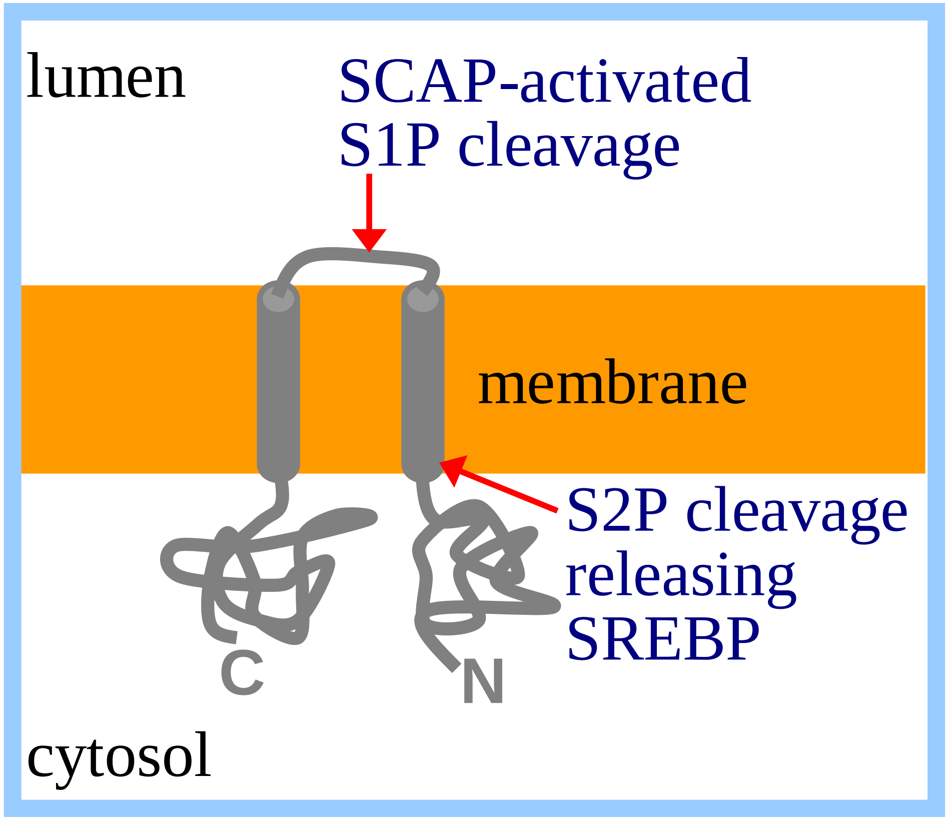
  This process, which is stimulated under conditions of nutritional or oxidative **stress**, involves interaction of proteins to be degraded with:

  - **Cytosolic chaperones** that unfold the proteins.
  - A lysosomal membrane **receptor** (LAMP-2A) that may provide a pathway across the membrane.
  - **Chaperones** in the lysosomal **lumen** that may assist with translocation across the membrane.

Intramembrane-cleaving proteases (**I-CLiPs**) cleave **regulatory proteins** such as transcription factors from membrane-anchored precursor proteins.

E.g., **precursors** of **SREBP** (sterol response element binding protein) transcription factors are integral proteins embedded in endoplasmic reticulum membranes.

Activation of **SREBP** involves its translocation to golgi membranes where sequential cleavage by 2 proteases releases to the cytosol a domain with transcription factor activity.



The released SREBP can then translocate to the cell nucleus to regulate transcription of genes for enzymes involved, e.g., in cholesterol synthesis.

S2P (site 2 protease, an **I-CLiP**) is a membrane-embedded **metalloprotease** that cleaves an α-helix of the SREBP precursor within the transmembrane domain.

Ubiquitin:

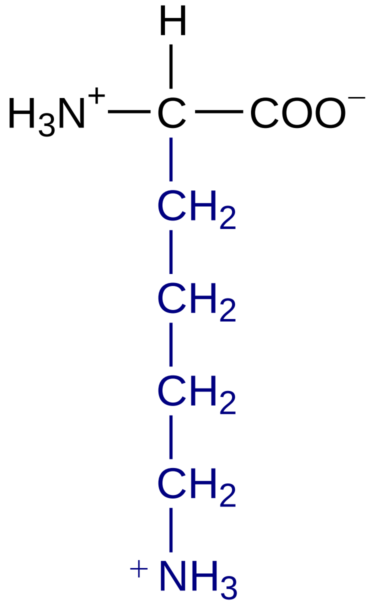
ubiquitin PDB 1TBE

Proteins are usually **tagged** for selective destruction in proteolytic complexes called **proteasomes** by covalent attachment of **ubiquitin**, a small, compact, highly conserved protein.

However, some proteins may be degraded by proteasomes without ubiquitination.

An isopeptide bond links the **terminal carboxyl** of **ubiquitin** to the **ε-amino** group of a **lysine** residue of a "condemned" protein.

lysine



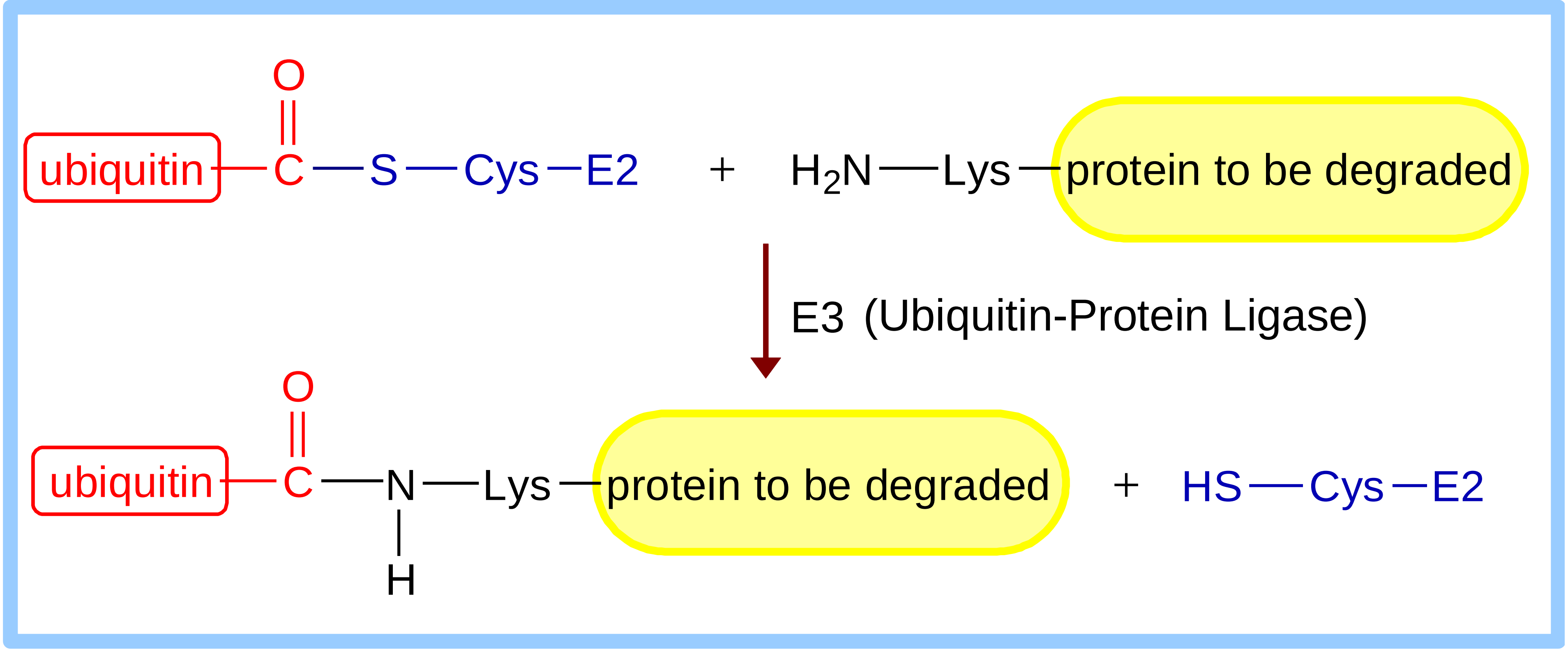
$$H_3N^+-\overset{\displaystyle H}{\underset{\displaystyle CH_2CH_2CH_2CH_2{}^+NH_3}{C}}-COO^-$$

The joining of ubiquitin to a condemned protein is **ATP-dependent**.

Three enzymes are involved, designated **E1**, **E2** & **E3**.

◆ Initially the **terminal carboxyl** group of **ubiquitin** is joined in a thioester bond to a cysteine residue on **Ubiquitin-Activating Enzyme** (**E1**). This is the ATP-dependent step.

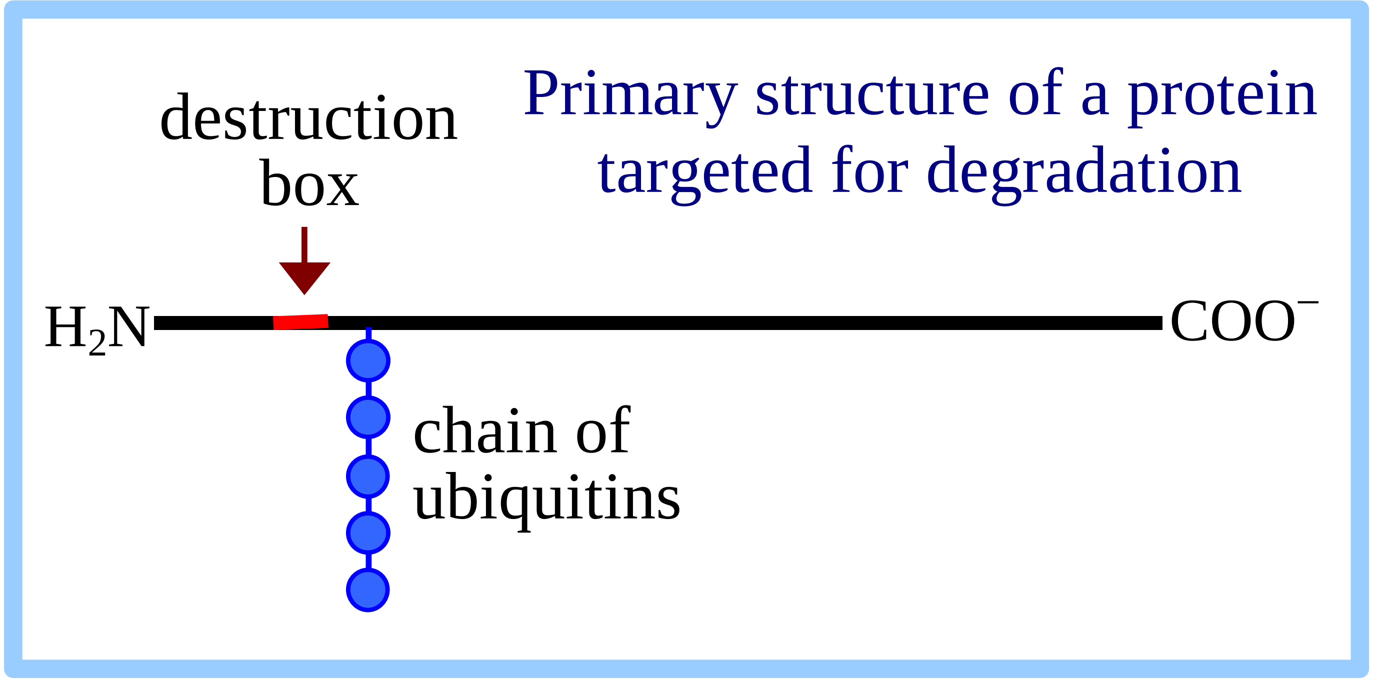
◆ The ubiquitin is then transferred to a sulfhydryl group on a **Ubiquitin-Conjugating Enzyme** (**E2**).



A **Ubiquitin-Protein Ligase** (**E3**) then promotes transfer of ubiquitin from E2 to the ε-amino group of a Lys residue of a protein recognized by that E3, forming an isopeptide bond.

There are **many distinct Ubiquitin Ligases** with differing substrate specificity.

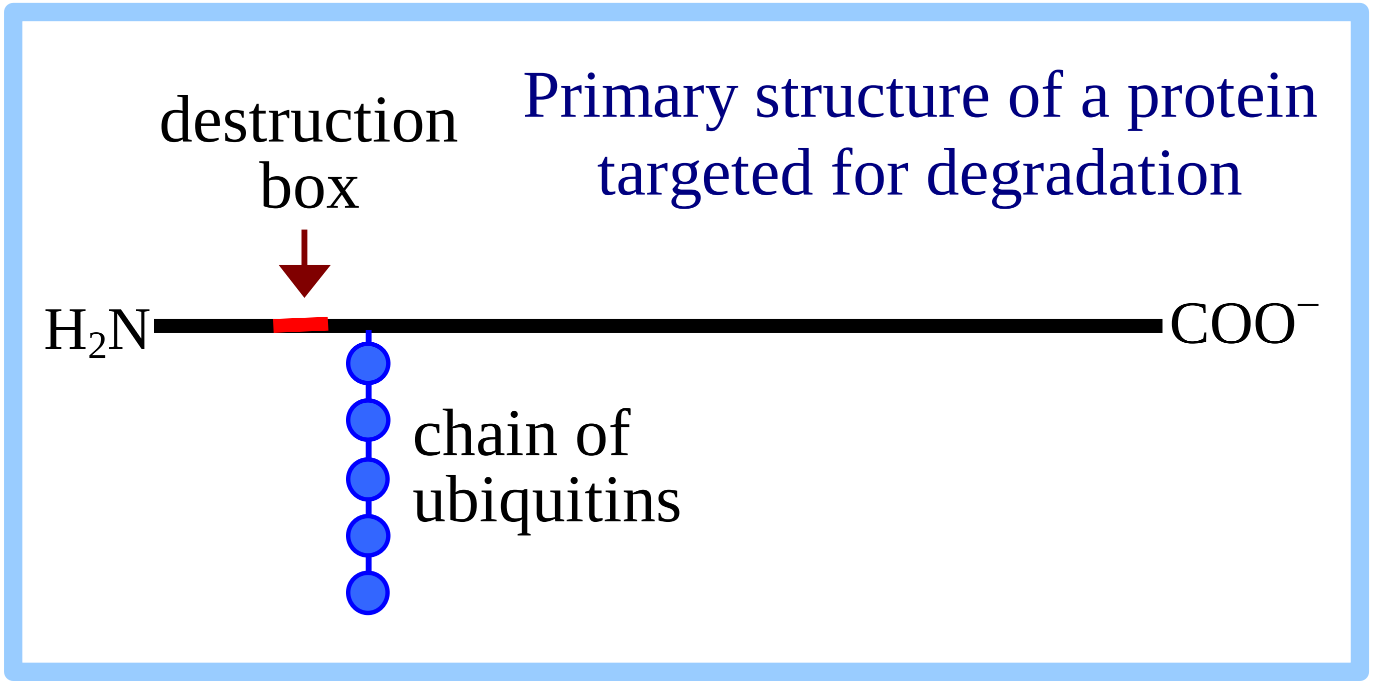
- One E3 is responsible for the **N-end** rule.
- Some are **specific** for particular proteins.



More ubiquitins are added to form a **chain of ubiquitins**.

The terminal carboxyl of each ubiquitin is linked to the ε-amino group of a lysine residue (Lys29 or Lys48) of the adjacent ubiquitin.

A chain of **4 or more ubiquitins** targets proteins for degradation in proteasomes. (Attachment of a single ubiquitin to a protein has other regulatory effects.)



Primary structure of a protein targeted for degradation

destruction box

H_2N COO^-

chain of ubiquitins

Some proteins (e.g., mitotic cyclins involved in cell cycle regulation) have a **destruction box** sequence recognized by a domain of the corresponding Ubiquitin Ligase.

Ubiquitin Ligases (**E3**) mostly consist of two families:

- Some Ubiquitin Ligases have a **HECT** domain containing a conserved **Cys** residue that participates in transfer of activated ubiquitin from E2 to a target protein.
- Some Ubiquitin Ligases contain a **RING finger** domain in which Cys & His residues are ligands to 2 **Zn**$^{++}$ ions.

  A RING (**R**eally **I**nteresting **N**ew **G**ene) finger is not inherently catalytic. It stabilizes a characteristic globular domain conformation that serves as a molecular scaffold for residues that interact with E2.

Regulation of ubiquitination:

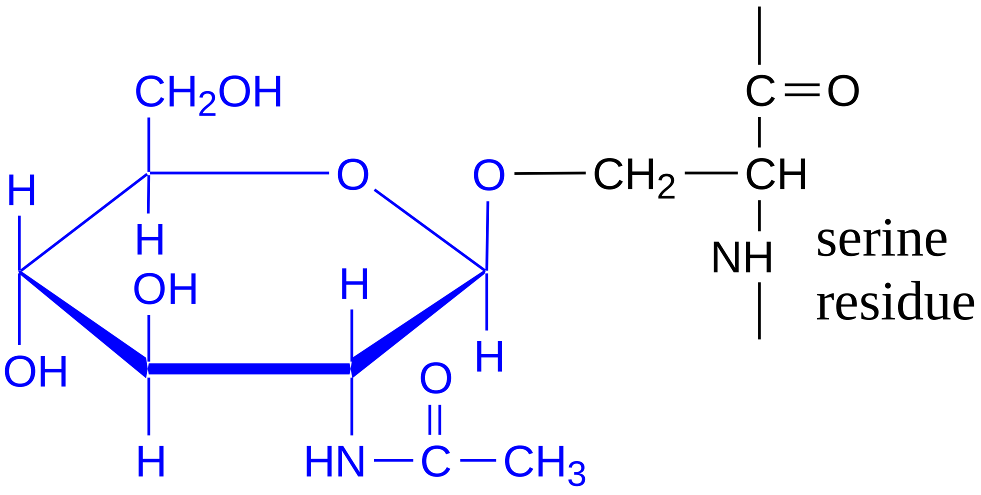
Some **proteins** regulate or facilitate ubiquitin conjugation.

Regulation by **phosphorylation** of some target proteins has been observed.

E.g., phosphorylation of **PEST** domains activates ubiquitination of proteins rich in the PEST amino acids.

Glycosylation of some PEST proteins with **GlcNAc** has the opposite effect, prolonging half-life of these proteins.

GlcNAc attachment increases with elevated extracellular glucose, suggesting a role as **nutrition sensor**.



β-D-*N*-acetylglucosamine

- A ubiquitin-like protein called **Nedd8** may be attached to **ubiquitin ligases** (**E3**) that have a "cullin" subunit including a RING finger domain.

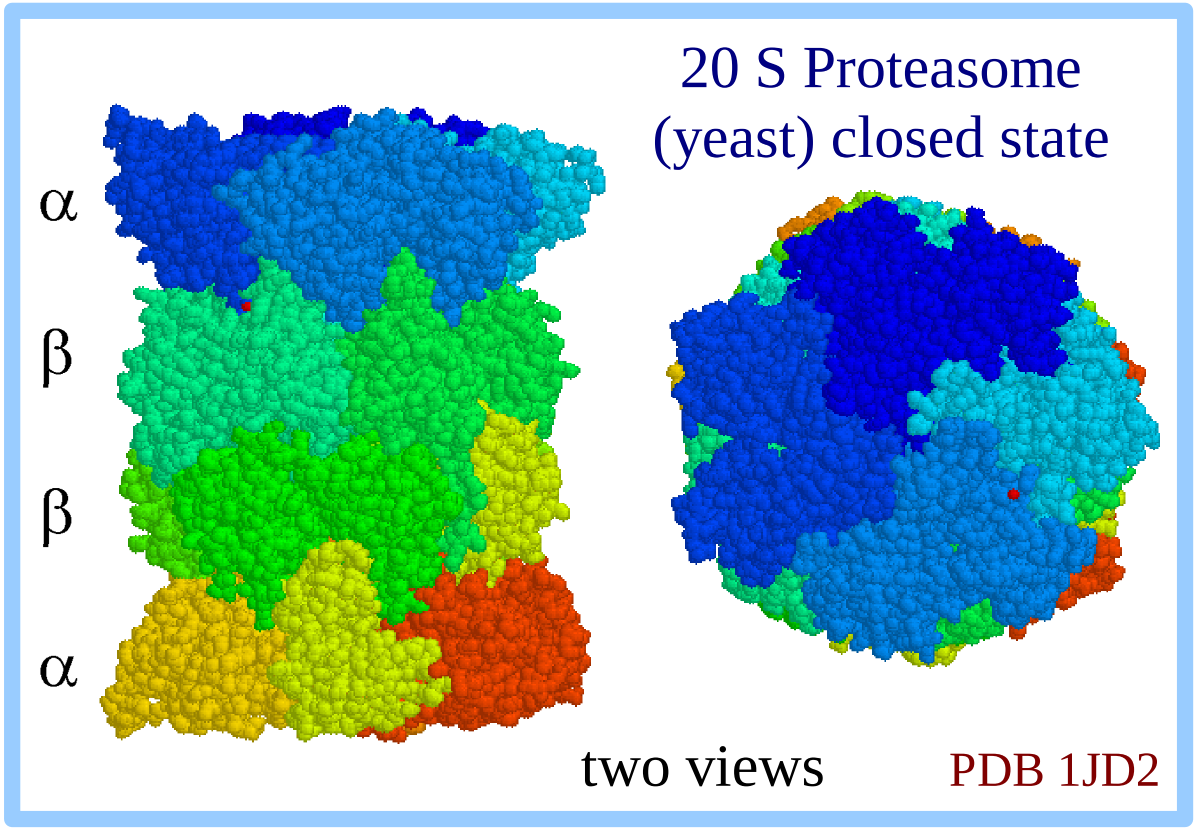
  De-neddylation (removal of the Nedd8 protein), catalyzed by a metalloprotease subunit of a complex called the COP9 signalosome, **activates** the **E3** ligases.

- Some disease-causing **viruses** target host cell proteins for degradation in the proteasome.

  They either **activate** a host cell **Ubiquitin Ligase** to ubiquitinate host proteins, or encode their own Ubiquitin Ligase.

Proteasomes:

Selective protein degradation occurs in the proteasome, a large protein complex in the nucleus & cytosol of eukaryotic cells.



20 S Proteasome (yeast) closed state

two views

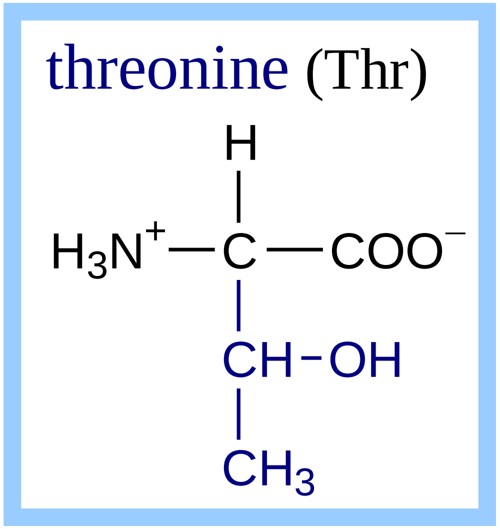
PDB 1JD2

The proteasome **core complex**, with a **20S** sedimentation coefficient, contains 2 each of 14 different polypeptides.

- 7 α-type proteins form each of the two α rings, at the ends of the cylindrical structure.
- 7 β-type proteins form each of the 2 central β rings.

1. One catalytic β-subunit has a **chymotrypsin-like** activity with preference for tyrosine or phenylalanine at the P1 (peptide carbonyl) position.
2. One has a **trypsin-like** activity with preference for arginine or lysine at the P1 position.
3. One has a **post-glutamyl** activity with preference for glutamate or other acidic residue at the P1 position.

Different **variants** of the 3 catalytic subunits, with different substrate specificity, are produced in cells of the **immune system** that cleave proteins for antigen display.



threonine (Thr)

$$H_3N^+\text{—}\overset{\displaystyle H}{\underset{\displaystyle \underset{\displaystyle CH_3}{CH\text{–}OH}}{C}}\text{—}COO^-$$

The proteasome hydrolases constitute a unique family of **threonine proteases**. A conserved N-terminal threonine is involved in catalysis at each active site.

The 3 catalytic β subunits are synthesized as **pre-proteins**. They are activated when the N-terminus is cleaved off, making threonine the N-terminal residue.

Catalytic threonines are exposed at the **lumenal surface**.

Proteasomal degradation of particular proteins is an essential mechanism by which **cellular processes** are **regulated**, such as cell division, apoptosis, differentiation and development.

E.g., progression through the **cell cycle** is controlled in part through regulated degradation of proteins called **cyclins** that activate **cyclin-dependent kinases**.

Several subunits of the **proteasome** are **glycosylated** with **GlcNAc** when extracellular glucose is high, leading to decreased intracellular proteolysis.

Conversely, under conditions of **low nutrition**, decreased modification by GlcNAc leads to **increased proteolysis**.

Thus **protein degradation is responsive to nutrition** via glycosylation of Ubiquitin Ligase & the proteasome itself.

Many **inhibitors** of proteasome protease activity are known, some of which are natural products and others experimentally produced.

E.g., **TMCs** are naturally occurring proteasome inhibitors.

- They bind with high affinity adjacent to active site threonines within the proteasome core complex.
- TMCs have a heterocyclic ring structure derived from modified amino acids.

Proteasome inhibitors cause **cell cycle arrest** and induction of **apoptosis** (programmed cell death) when added to rapidly dividing cells.

- The potential use of proteasome inhibitors in treating **cancer** is being investigated.

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SProtP: A Web Server to Recognize Those Short-Lived Proteins Based on Sequence-Derived Features in Human Cells

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Abstract

Protein turnover metabolism plays important roles in cell cycle progression, signal transduction, and differentiation. Those proteins with short half-lives are involved in various regulatory processes. To better understand the regulation of cell process, it is important to study the key sequence-derived factors affecting short-lived protein degradation. Until now, most of protein half-lives are still unknown due to the difficulties of traditional experimental methods in measuring protein half-lives in human cells. To investigate the molecular determinants that affect short-lived proteins, a computational method was proposed in this work to recognize short-lived proteins based on sequence-derived features in human cells. In this study, we have systematically analyzed many features that perhaps correlated with short-lived protein degradation. It is found that a large fraction of proteins with signal peptides and transmembrane regions in human cells are of short half-lives. We have constructed an SVM-based classifier to recognize short-lived proteins, due to the fact that short-lived proteins play pivotal roles in the control of various cellular processes. By employing the SVM model on human dataset, we achieved 80.8% average sensitivity and 79.8% average specificity, respectively, on ten testing dataset (TE1-TE10). We also obtained